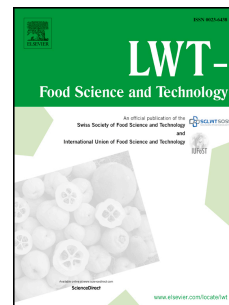


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CRediT authorship contribution statement

**Eva Achata:** Investigation, Data Curation, Formal analysis, Visualization, Writing - Original draft, **Marcia Oliveira:** Investigation, Data Curation **Carlos Esquerre:** Software, Writing - Review & Editing **Brijesh Tiwari:** Conceptualization, Methodology, Resources **Colm O'Donnell:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition

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# Visible and NIR hyperspectral imaging and chemometrics for prediction of microbial quality of beef *Longissimus dorsi m.* under simulated normal and abuse storage conditions

Achata EM<sup>1</sup>, Oliveira M<sup>2</sup>, Esquerre CA<sup>1</sup>, Tiwari BK<sup>2</sup>, O'Donnell CP<sup>1</sup>

<sup>1</sup>School of Biosystems and Food Engineering, University College Dublin, Ireland

<sup>2</sup>Department of Food Chemistry & Technology, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

## Abstract

There is a need to develop a rapid technique to provide real time information on the microbial load of meat along the supply chain. Hyperspectral imaging (HSI) is a rapid, non-destructive technique well suited to food analysis applications. In this study, HSI in both the visible and near infrared spectral ranges, and chemometrics were studied for prediction of the bacterial growth on beef *Longissimus dorsi m.* (*LD*) under simulated normal (4 °C) and abuse (10 °C) storage conditions. Total viable count (TVC) prediction models were developed using partial least squares regression (PLS-R), spectral pre-treatments, band selection and data fusion methods. The best TVC prediction models developed for storage at 4 (RMSE<sub>p</sub> 0.58 log CFU/g, RPD<sub>p</sub> 4.13, R<sup>2</sup><sub>p</sub> 0.96), 10 °C (RMSE<sub>p</sub> 0.97 log CFU/g, RPD<sub>p</sub> 3.28, R<sup>2</sup><sub>p</sub> 0.94) or at either 4 or 10 °C (RMSE<sub>p</sub> 0.89 log CFU/g, RPD<sub>p</sub> 2.27, R<sup>2</sup><sub>p</sub> 0.86) were developed using high-level data fusion of both spectral regions. The use of appropriate spectral pre-treatments and band selection methods was key for robust model development. This study demonstrated the potential of HSI and chemometrics for real time monitoring to predict microbial growth on *LD* along the meat supply chain.

Key words: Hyperspectral imaging, chemometrics, TVC prediction, data fusion, meat.

## 1. Introduction

The *Longissimus dorsi m. (LD)* of beef is highly valued by consumers and is normally aged to increase tenderness, juiciness and flavour. Meat processors generally age *LD* for 28 days or longer to improve tenderness and flavour. However colour and microbial load are also affected which potentially impacts product safety and shelf life (Borch, Kant-Muermans, & Blixt, 1996; Vitale, Pérez-Juan, Lloret, Arnau, & Realini, 2014). The design and application of quality and safety assurance systems are based on thorough risk analysis and control of critical parameters through the entire life cycle of meat products including raw material selection and control during processing and distribution. The temperature profiles during transportation and at retail level are not within the direct control of meat processors and may exceed recommended temperatures. Lack of temperature control from retail to the time of preparation and consumption may also be an issue. In southern European countries 30% of refrigerated foods were reported to be stored above 10 °C in retail cabinets and household refrigerators (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

Current microbiological methods are not suitable for real time monitoring of microbial contamination of meat. The traditional plate count technique is the most commonly used method to monitor microbial load. However, it requires time consuming sample preparation and analysis. The total viable count (TVC) method is an important microbiology indicator for quality and safety evaluation of meat (Lytou, Panagou, & Nychas, 2016). The initial microbial load of meat after processing, storage temperature, pH and relative humidity are the main factors influencing microbial load throughout the supply chain. Enzyme-linked immune absorbent assay (ELISA), gene analysis-based methods such as polymerase chain reaction (PCR) and DNA sequencing are also employed for microbial contamination detection (Si et al., 2016) but are not suited to online analysis.

Visible (VIS) and near-infrared (NIR) spectroscopy are rapid non-destructive techniques widely used in environmental, pharmaceutical, fuel and food analysis applications. The VIS

and NIR spectral regions range from 380 - 740 nm and 700 - 2500 nm respectively.

Spectroscopic sensors usually acquire spectra from a limited field of view which limits their applicability for rapid safety analysis of large volume batches or analysis of heterogeneous samples such as meat products (Millar, Moss, & Stevenson, 1996).

Hyperspectral imaging (HSI) is a rapid analytical tool for non-destructive measurement of food quality and safety. HSI integrates traditional imaging and spectroscopy to acquire both spatial and spectral information from samples. Each pixel in a hyperspectral image contains the spectrum of that specific position, i.e. the light-absorbing and/or scattering properties of the spatial region represented, which can be used to characterise the composition of that particular pixel (Gowen, O'Donnell, Cullen, Downey, & Frias, 2007; Kamruzzaman, Makino, & Oshita, 2016). HSI techniques can be employed at different points along meat distribution chains. HSI has been studied to predict microbial growth on fresh beef meat using the VIS range (Peng et al., 2011; Tao, Peng, Gomes, Chao, & Qin, 2015). However, no studies have been reported to date on the use of HSI in the NIR spectral range to predict microbial growth on fresh beef.

Chemometric methods are employed to develop prediction models from HSI data. Partial least squares regression (PLS-R) may be used to predict unknown concentrations and generate prediction maps to estimate spatial distributions of components in samples (Gowen, Burger, Esquerre, Downey, & O'Donnell, 2014). Spectral pre-treatments are used to correct for the effects of natural variability in the shape and size of samples, light scattering and differences in the effective path length in spectral data, which can present difficulties in the application of HSI for quality assessment (Esquerre, Gowen, Burger, Downey, & O'Donnell, 2012). Band selection methods have been demonstrated to improve the performance of regression models and to reduce the processing times required to evaluate HSI data by selecting the most informative bands. The variable importance projection method (VIP), the selectivity ratio method (SR) and the ensemble Monte Carlo variable selection method

(EMCVS) have been demonstrated to be reliable band selection methods for HSI data (Achata, Inguglia, Esquerre, Tiwari, & O'Donnell, 2019; Farrés, Platikanov, Tsakovski, & Tauler, 2015).

Data fusion combines information from different sources to produce a more reliable and accurate model or information. Three levels of data fusion may be employed i) low level (data-level) fusion, where data from all sources are properly transformed and concatenated for model development, ii) mid-level (feature-level) fusion, where variable selection or feature extraction is applied to each data source before the extracted features are combined; iii) and high-level (decision-level) fusion where a model is constructed for each data source separately and their predictions combined thereafter (Liu & Brown, 2004). Data fusion has been studied to detect volatile basic nitrogen (TVB-N) content in chicken meat using a colorimetric sensor and a VIS system (Khulal, Zhao, Hu, & Chen, 2017).

The objective of this study was to investigate the potential of HSI and chemometrics for the prediction of the microbial quality of beef under simulated normal (4 °C ) and abuse (10 °C) storage conditions.

## **2. Materials and methods**

### **2.1. Sample preparation**

*LD* samples ( $n = 104$ ) from 9 cattle (denoted S1 to S9) of ca. 25 mm thickness were obtained from local supermarkets and a meat processing facility. The samples were placed in sealed food containers and randomly assigned for storage at either 4 °C ( $n = 53$ ) for 360 hours or at 10 °C ( $n = 51$ ) for 168 hours. Three randomly selected samples (from 3 cattle) were removed from storage and scanned using a visible short wave near infrared (VIS-SWNIR) and an NIR HSI systems. The TVC of samples was measured after scanning using the ISO 48833-1:2013 methodology (ISO, 2013). Briefly 25 g of each sample was suspended in 225 ml of buffered peptone water (BPW, Oxoid, Hampshire, England) and aseptically homogenized in a

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stomacher (Star-Blender LB 400, VWR) for 2 min. Further decimal dilutions were made with maximum recovery diluent (MRD, HyServe, Germany). Three replicates were assessed per sample at each sampling time. Reported populations represent the mean of three values.

## **2.2. Hyperspectral images**

Hyperspectral images of the *LD* samples were obtained using a VIS-SWNIR HSI system (400 – 1000 nm) and an NIR HSI system (880 – 1720 nm) (DV Optics, Padova, Italy). Calibration of both HSI systems was performed as outlined by Achata, Esquerre, O'Donnell, and Gowen (2015). The acquired hypercubes were saved in ENVI formatted files and imported into MATLAB (The MathWorks Inc., Natick, MA, USA) for further spatial and spectral data pre-processing and chemometric analysis, using in-house developed functions and scripts.

### **2.2.1. VIS-SWNIR HSI spatial and spectral pre-processing**

The noise present at both ends of the spectra was removed by trimming the spectral range to 445 - 970 nm. The background was removed using a mask created using the ratio between bands 80 (840 nm) and 20 (540 nm) and removing pixels with a ratio value  $> 1.5$ . To improve the signal-to-noise ratio (SNR) and reduce processing times and data storage required,  $2 \times 2$  binning was performed on the obtained hypercubes of 1000 x 580 pixel image with 106 spectral bands, resulting in hypercubes of 500 x 290 pixel image with 106 spectral bands. The binned 3-dimensional hypercubes were unfolded into matrices of pixel spectra (14500 pixel x 106 spectral bands) to facilitate algorithm development. The mean reflectance (*R*) spectra of each masked sample was calculated and smoothed using the Savitzky - Golay (SG) 5 points second order polynomial method prior to chemometric analysis (Savitzky & Golay, 1964).

## 2.2.2. NIR-HSI spatial and spectral pre-processing

The noise present at both ends of the spectra was removed by trimming the spectral range to 957 - 1664 nm. Dead pixels and spikes were removed by replacing the affected values with the mean values of adjacent bands in the same spectrum. The background was removed using a mask which was created with the ratio between bands 90 (1580 nm) and 20 (1090 nm), removing pixels with a ratio value  $> 0.65$ . Images were segmented using the pixel ratio between bands 37 (1209 nm) and 43 (1251 nm) to remove fat and connective tissue (ratio value  $> 0.7$ ). The 3-dimensional hypercubes (500 x 320 pixel image with 102 bands) were unfolded into matrices of pixel spectra (160000 pixel x 102 bands). The mean reflectance spectra of each segmented sample was calculated and smoothed using the Savitzky - Golay (SG) 5 points second order polynomial method prior to chemometric analysis.

## 2.3. Chemometric analysis

### 2.3.1. PCA

PCA (not reported) was carried out to investigate the relationships between storage temperature over time and spectral data, and to identify potential outliers using the Hotelling  $T^2$  statistic. A sample was considered as an outlier if the  $T^2$  value was  $> T^2_{crit} = A \times F_{(0.05, A, n - A)} \times (n-1)/(n-A)$ , where  $A$  is the number of significant components,  $n$  is the number of spectra in the dataset and  $F_{(0.05, A, n - A)}$  is the  $F$  statistic (with  $\alpha = 0.05$ ,  $A$  and  $n - A$  degrees of freedom).

### 2.3.2. PLS-R

PLS regression (Wold, Sjöström, & Eriksson, 2001) models were developed to predict TVC of samples using HSI data, spectral pre-treatments, band selection and data fusion methods.



Spectral data sets were split into calibration and validation sets to develop and validate the prediction models. Smoothed mean spectral data from 4 randomly selected samples (S1, S2, S4 and S6 (n=69)) was used for calibration and samples (S3 and S5) were used to validate the models (n=35). Predictions models were evaluated using the:

- i) Smoothed mean spectral data of samples stored at 4 °C (n = 53)
- ii) Smoothed mean spectral data of samples stored at 10 °C (n = 51)
- iii) Smoothed mean spectral data of samples stored at either 4 or 10 °C (n = 104).

The number of latent variables (LV) were selected by analysis of the root mean square error of ten-fold cross-validation (RMSE<sub>CV</sub>) presented in Eq. (1) and roughness of the regression vector.

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad (1)$$

$y_i$  and  $\hat{y}_i$  are the measured and predicted values of the microbial counts respectively.

### 2.3.3. Spectral pre-treatments

Standard normal variate (SNV), median scaled (MS), Savitzky-Golay 7 points second order polynomial first derivative (FD), Savitzky-Golay 7 points second order polynomial second derivative (SD), Savitzky-Golay 11 points fourth order polynomial third derivative (TD), linear detrending second-order polynomial (LD), asymmetric least squares (AsLs) (Barnes, Dhanoa, & Lister, 1989; Boelens, Eilers, & Hankemeier, 2005; Engel et al., 2013; Savitzky & Golay, 1964) and all combinations of any two spectral pre-treatments were applied. The Savitzky-Golay derivative (FD, SD or TD) window length and polynomial order were selected by preliminary tests on 10 randomly selected spectra.

### 2.3.4. Band selection

The VIP (Eriksson, Hermens, Johansson, Verhaar, & Wold, 1995; Wold et al., 2001), SR (Rajalahti et al., 2009) and the EMCVS (Esquerre, Gowen, O'Gorman, Downey, & O'Donnell, 2017) band selection methods were evaluated and compared with and without spectral pre-treatments.

The performance of the regression models was assessed using the root mean square error (RMSE), the ratio of standard deviation of the reference data of the calibration set and the RMSE (RPD) and the coefficient of determination ( $R^2$ ) for calibration (C), cross-validation (CV) and prediction (P) sets (Eq. 2-4). The best model was selected based on the number of latent variables, selected wavebands and the geometric mean of the RPD values from calibration, cross-validation, and prediction sets. Prediction models developed for complex matrices can be classified as excellent (RPD > 4.1), very good (RPD 3.5 – 4.0), good (RPD 3.0 – 3.4), fair (RPD 2.5 – 2.9) and poor (RPD 2.0 – 2.4) (Williams, 2014).

$$RPD = \frac{s_{y_{cal}}}{SEP} \quad (2)$$

$$SEP = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i - bias)^2}{n-1}} \quad (3)$$

$$R^2 = \left( \frac{\sum_{i=1}^n (y_i \hat{y}_i) - n \bar{y} \bar{\hat{y}}}{\sqrt{\sum_{i=1}^n y_i^2 - n \bar{y}^2} \sqrt{\sum_{i=1}^n \hat{y}_i^2 - n \bar{\hat{y}}^2}} \right)^2 \quad (4)$$

Where the *bias* is the average difference between reference value and predicted value (Eq. 5).

$$bias = \frac{\sum_{i=1}^n (y_i - \hat{y}_i)}{n} \quad (5)$$

### 2.3.5. Data fusion

Prediction models for TVC of samples were developed for low level (LL), medium level (ML) and high-level (HL) data fusion of VIS-SWNIR and NIR HSI data. For the LL data fusion, the spectral data of both systems was concatenated before model development. For the ML data fusion, the selected spectral bands (obtained by the band selection method that achieved the best performance for each system) were combined. When the variance of the

pre-treated VIS-SWNIR and NIR HSI data was different, each data block was scaled to unit variance. The calibration, validation and prediction sets were scaled using the inverse of the standard deviation of the calibration set (Forshed, Idborg, & Jacobsson, 2007). For the HL data fusion, prediction models were developed by averaging the predictions of the best performing models identified for each system.

### 3. Results and discussion

#### 3.1. TVC of samples

The TVC of *LD* samples during storage at 4 °C and 10 °C are presented in Table 1. TVC values increased from 3.4 to 14.1 log CFU/g over 360 h storage at 4 °C and increased from 3.4 to 13.1 log CFU/g over 168 h storage at 10 °C. Initial TVC values (day 0) varied according to the origin of the *LD* samples. *LD* S1 to S6 samples were purchased from local supermarkets and had initial TVC values > 6 log CFU/g. However, *LD* samples purchased directly from the meat processing facility (S7 to S9) had lower initial TVC values. *LD* S9 (non aged) samples had the lowest TVC values (3.4 log CFU/g), while samples from the *LD* S7 and S8 which were both aged for 28 days had TVC values of ca. 5.3 log CFU/g.

Previous studies reported that *LD* samples with TVC values < 7 log CFU/g are acceptable, and samples with values > 7 log CFU/g are spoiled (Tao et al., 2015). Moreover, the presence of slime and discolouration has been reported for meat samples with TVC values > 7 log CFU/g (Bell & Garout, 1994).

#### 3.2. Spectral characteristics of *LD* samples

PCA analysis revealed the presence of one outlier in the VIS-SWNIR spectra of the *LD* samples stored at 4 °C, which was removed from the dataset. No outliers were identified in the NIR spectra. Figs. 1 and 2 show the spectral variations between samples during storage at 4 and 10 °C respectively. The SD + AsLs pre-treated log (1/R) NIR spectra were used for

TVC prediction at 4 °C, whereas the SD+MS pre-treated reflectance VIS-SWNIR spectra were selected for TVC prediction at 10 °C. Spectral shifts are more apparent during storage due to changes in physical characteristics, chemical composition and microbial activity. Spoiled samples exhibited broader absorption bands compared to unspoiled samples.

Absorbance peaks observed at 1076 and 1342 nm in Fig. 1 may be related to the C-H stretching of the first and second overtone regions respectively, and the peak at 1580 nm may be related to the 1<sup>st</sup> overtone of O-H stretching (glucose) (Osborne, Fearn, & Hindle, 1993). The selected bands highlighted in Fig.1 provide complementary information on the samples and are related to the 2<sup>nd</sup> overtone of O-H stretching (978 nm) of water and the 1<sup>st</sup> overtone of N-H stretching (1496 nm) of protein. Similar spectral bands were observed by Barbin, ElMasry, Sun, Allen, and Morsy (2013) for pork samples. The observed differences between spoiled and fresh meat may relate to the presence of protein, free amino acids, amines or nitrogen bearing substances and their interactions with water. Such observations are consistent with the proteolytic changes which occur during microbial spoilage (Atanassova, Veleva, & Stoyanchev, 2018).

Fig. 2 shows the characteristic peaks of the of oxymyoglobin ( $\text{MbFe}^{\text{II}}\text{O}_2$ ) at 545 and 580 nm (Achata et al., 2019; Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013; Millar et al., 1996). These bands are prominent at the start of storage and increase in intensity after 24 h storage at 10 °C ( $\text{TVC} < 7.3 \log \text{CFU/g}$ ). The intensity of these bands decreases after 48 h corresponding to TVC values  $> 7.5 \log \text{CFU/g}$ . These changes may correspond to a decrease in the concentration of red pigments due to microbial growth during storage. The prominent peak at 765 nm may be related to the 3<sup>rd</sup> overtone C-H stretching. The selected bands highlighted in Fig. 2 provide complementary information on the samples and may be related to the 3<sup>rd</sup> overtone of C-H stretching (750 - 780 nm) (Osborne et al., 1993).

### 3.3. TVC prediction models

SD, SNV and the combination of SD+LD, and SNV+SD were identified as the best performing spectral pre-treatments after evaluating 50 combinations for each band selection method (Appendix 1). Models developed using the variable selection approach were compared with the best models developed using the full spectral range for both the VIS-SWNIR and NIR HSI spectral regions.

The best performing PLS-R model developed to predict TVC during storage at 4 °C (Table 2) was developed using the NIR-HSI data and EMCVS of the SD+AsLs pre-treated  $\log(1/R)$  spectra (17 selected bands, LV 7, RMSEP 0.81 log CFU/g, RPD<sub>P</sub> 3.09,  $R^2_P$  0.95). Lower coefficients of determination for the prediction of pork meat TVC were obtained by Barbin et al. (2013). Fig. 3a shows the predicted versus measured TVC values for *LD* samples stored at 4 °C obtained with the SD+AsLs pre-treated  $\log(1/R)$  spectra. HL data fusion improved the performance of the prediction models (RMSEP 0.58 log CFU/g, RPD<sub>P</sub> 4.13,  $R^2_P$  0.96) compared to those obtained with LL data fusion, ML data fusion and the best models selected for the VIS-SWNIR and NIR HSI spectral data (Table 5).

The best performing PLS-R model developed to predict TVC during storage at 10 °C (Table 3) was developed using the VIS-SWNIR - HSI data and EMCVS of the SD+MS pre-treated reflectance spectra (46 selected bands, LV 6, RMSEP 0.96 log CFU/g, RPD<sub>P</sub> 3.32,  $R^2_P$  0.94). Fig.3b. shows the predicted versus measured TVC values for *LD* samples stored at 10 °C obtained with the SD+MS pre-treated reflectance spectra. The use of derivative pre-treatments of VIS-SWNIR spectra has been reported to accentuate the differences in myoglobin spectra (Millar et al., 1996) by removing baseline offsets and decreasing scattering effects (Esquerre et al., 2012) as observed in Fig. 2. LL and HL data fusion yielded good prediction models, comparable to those obtained with the VIS-SWNIR data and better than the models developed using ML data fusion (Table 5).

The best performing PLS-R models developed to predict TVC for samples stored at either 4 or 10 °C (Table 4) were developed using the VIS-SWNIR data and EMCVS of the SNV+SD pre-treated reflectance spectra (8 selected bands, LV 4, RMSEP 0.95 log CFU/g, RPD<sub>P</sub> 2.10, R<sup>2</sup><sub>P</sub> 0.85). Improved data fusion PLS-R models were developed using the LL and HL data fusion showed in Table 5 (RMSEP 0.87 log CFU/g, RPD<sub>P</sub> 2.27, R<sup>2</sup><sub>P</sub> 0.88, and RMSEP 0.89 log CFU/g, RPD<sub>P</sub> 2.27, R<sup>2</sup><sub>P</sub> 0.86 respectively). Fig. 3c shows the predicted versus measured TVC values of *LD* stored at either 4 or 10 °C obtained using HL data fusion of both spectral regions.

Selected TVC prediction maps built using the best prediction model developed for storage at 10 °C (SD+MS on the reflectance VIS-SWNIR spectra) are shown in Fig. 4.

Good and excellent TVC prediction models were obtained for beef *LD* samples stored at 4 °C for both the NIR spectral range and HL data fusion of the VIS-SWNIR and NIR spectral regions respectively based on the RPD prediction model performance classifications reported by Williams (2014) for complex matrices. Good TVC prediction models were also obtained for samples stored at 10 °C using the VIS-SWNIR spectral range and HL data fusion of both spectral regions. However poor TVC prediction models were obtained for samples stored at either 4 °C or 10 °C using the VIS-SWNIR spectral range and HL data fusion of both spectral regions. In all cases EMCVS outperformed the other band selection methods evaluated. Combinations of SD, SNV and LD spectral pre-treatments also improved regression model development. Data fusion approaches improved prediction model performance in all cases. This is in agreement with the study reported by (Li, Chen, Zhao, and Wu (2015)) who reported that superior regression models can be obtained using data fusion and appropriate band selection methods.

#### 4. Conclusions

Excellent ( $\text{RMSE}_p$  0.58 log CFU/g,  $\text{RPD}_p$  4.13,  $R^2_p$  0.96) and good ( $\text{RMSE}_p$  0.97 log CFU/g,  $\text{RPD}_p$  3.28,  $R^2_p$  0.94) TVC prediction models were developed for beef *LD* samples stored at 4 °C and 10 °C respectively, using the HL data fusion of the two spectral regions. Prediction models were successfully developed using spectral pre-treatments, the full spectral range, selected bands and data fusion of both VIS-SWNIR and NIR spectral regions to predict the TVC of *LD* samples with low prediction errors.

The application of SD and SNV spectral pre-treatments improved the performance of the developed models using both spectral ranges and on selected bands. EMCVS improved the performance of the TVC prediction models developed compared with the full spectral range and outperformed VIP and SR methods. Data fusion approaches improved prediction model performance in all cases, HL data fusion yielded the best TVC prediction models ( $\text{RMSE}_p$  0.89 log CFU/g,  $\text{RPD}_p$  2.27,  $R^2_p$  0.86) for beef samples stored at both 4 °C and 10 °C. Appropriate band selection was key for robust model development. This study demonstrated the potential of HSI and chemometrics as a rapid analytical tool for monitoring meat microbial quality along the supply chain.

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Appendix 1. Performance of the TVC PLS-R models developed using VIS-SWNIR (445 - 970 nm ) data from beef *LD* samples stored at 4 °C. PLS full spectral range on reflectance (R) and logarithmic transformed (log(1/R)) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre-treatment	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	106	7	0.91	2.73	0.87	1.48	1.69	0.68	1.72	1.20	0.72
		SNV	106	7	0.84	2.97	0.89	1.20	2.08	0.78	1.62	1.37	0.78
		SD	100	8	0.61	4.09	0.94	1.08	2.30	0.82	2.16	1.21	0.71
		SD+LD	100	8	0.61	4.10	0.94	1.09	2.30	0.82	2.05	1.23	0.71
		SNV+SD	100	6	0.79	3.15	0.90	1.34	1.86	0.74	1.35	1.61	0.81
	Log(1/R)	None	106	3	1.27	1.96	0.74	1.59	1.56	0.60	1.79	1.20	0.58
		SNV	106	5	0.89	2.79	0.87	1.16	2.16	0.78	1.45	1.45	0.75
		SD	100	6	0.63	3.94	0.94	1.06	2.37	0.82	1.93	1.22	0.67
		SD+LD	100	6	0.64	3.87	0.93	1.05	2.39	0.82	1.83	1.25	0.67
		SNV+SD	100	4	0.82	3.06	0.89	1.24	2.04	0.76	1.45	1.44	0.75
VIP	R	None	7	2	1.27	1.97	0.74	1.41	1.77	0.68	1.79	1.33	0.66
		SNV	27	6	0.97	2.58	0.85	1.20	2.07	0.77	1.80	1.25	0.75
		SD	28	7	0.94	2.65	0.86	1.38	1.81	0.70	1.78	1.25	0.70
		SD+LD	33	10	0.51	4.89	0.96	0.90	2.79	0.87	2.50	1.40	0.74
		SNV+SD	18	11	0.46	5.47	0.97	0.79	3.18	0.90	1.31	1.92	0.81
	Log(1/R)	None	4	3	1.21	2.06	0.76	1.39	1.79	0.70	1.77	1.25	0.60
		SNV	17	11	0.84	2.98	0.89	1.21	2.07	0.77	1.65	1.33	0.71
		SD	18	6	0.79	3.16	0.90	1.13	2.21	0.80	1.72	1.30	0.68
		SD+LD	15	3	1.10	2.27	0.81	1.28	1.95	0.74	1.34	1.66	0.76
		SNV+SD	13	8	0.55	4.51	0.95	0.70	3.56	0.92	1.38	1.86	0.80

SR	R	None	14	8	0.96	2.59	0.85	1.39	1.79	0.71	1.66	1.35	0.76
		SNV	1	1	1.78	1.40	0.49	1.86	1.34	0.44	2.21	1.31	0.60
		SD	7	4	1.01	2.47	0.84	1.27	1.96	0.74	1.64	1.35	0.67
		SD+LD	3	3	1.08	2.30	0.81	1.27	1.97	0.74	1.55	1.42	0.73
		SNV+SD	1	1	1.34	1.87	0.71	1.43	1.74	0.67	1.84	1.28	0.66
	Log(1/R)	None	11	8	0.95	2.64	0.86	1.29	1.94	0.74	1.44	1.59	0.75
		SNV	15	6	1.15	2.16	0.79	1.50	1.67	0.64	1.36	1.63	0.74
		SD	1	1	1.26	1.97	0.74	1.34	1.87	0.71	2.00	1.10	0.48
		SD+LD	5	3	1.09	2.28	0.81	1.26	1.98	0.75	1.96	1.23	0.56
		SNV+SD	7	5	1.00	2.48	0.84	1.30	1.92	0.73	1.95	1.64	0.76
EMCVS	R	None	6	3	1.05	1.94	0.73	1.18	1.72	0.67	2.07	1.10	0.57
		SNV	8	6	0.93	2.69	0.86	1.16	2.16	0.79	1.72	1.30	0.79
		SD	5	3	0.82	3.06	0.89	0.94	2.66	0.86	1.84	1.33	0.70
		SD+LD	10	5	0.74	3.38	0.91	0.89	2.81	0.87	2.11	1.25	0.68
		SNV+SD	7	3	0.90	2.77	0.87	1.02	2.44	0.83	1.52	1.47	0.72
	Log(1/R)	None	43	8	0.85	2.93	0.88	1.18	2.12	0.79	1.54	1.43	0.71
		SNV	9	3	1.02	2.45	0.83	1.13	2.20	0.79	1.64	1.44	0.71
		SD	10	7	0.69	3.63	0.92	0.89	2.82	0.87	1.42	1.58	0.73
		SD+LD	8	5	0.70	3.54	0.92	0.83	3.01	0.89	1.56	1.48	0.72
		<b>SNV+SD</b>	<b>6</b>	<b>5</b>	<b>0.88</b>	<b>2.83</b>	<b>0.88</b>	<b>1.03</b>	<b>2.42</b>	<b>0.83</b>	<b>1.17</b>	<b>1.88</b>	<b>0.83</b>

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for 4 °C is highlighted in bold.

Appendix 2. Performance of the TVC PLS-R models developed using VIS-SWNIR (445 - 970 nm ) data from beef *LD* samples stored at 10 °C. PLS full spectral range on reflectance (R) and logarithmic transformed (log(1/R)) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre-treatment	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	106	8	0.37	5.84	0.97	0.64	3.38	0.92	1.38	1.66	0.75
		SNV	106	7	0.39	5.59	0.97	0.63	3.45	0.92	1.39	1.56	0.74
		SD	100	6	0.66	3.27	0.91	1.00	2.17	0.79	1.18	1.67	0.75
		SD+LD	100	5	0.69	3.13	0.90	1.02	2.12	0.78	1.15	1.72	0.76
		SNV+SD	100	4	0.60	3.59	0.92	0.81	2.68	0.86	0.80	2.63	0.89
	Log(1/R)	None	106	8	0.42	5.14	0.96	0.71	3.05	0.89	1.14	2.48	0.88
		SNV	106	7	0.42	5.13	0.96	0.69	3.15	0.90	1.02	2.32	0.86
		SD	100	4	0.64	3.41	0.91	0.84	2.58	0.85	1.21	1.84	0.80
		SD+LD	100	5	0.53	4.11	0.94	0.74	2.92	0.88	1.16	2.02	0.82
		SNV+SD	100	5	0.54	4.05	0.94	0.73	2.98	0.89	1.01	2.31	0.87
VIP	R	None	20	8	0.48	4.49	0.95	0.73	2.97	0.89	1.06	1.99	0.84
		SNV	37	8	0.41	5.29	0.96	0.63	3.42	0.91	1.58	1.27	0.72
		SD	33	5	0.76	2.86	0.88	1.03	2.10	0.78	1.09	1.77	0.81
		SD+LD	27	6	0.72	3.03	0.89	1.05	2.06	0.76	0.91	1.97	0.82
		SNV+SD	9	4	0.65	3.34	0.91	0.80	2.72	0.87	0.92	2.27	0.87
	Log(1/R)	None	15	6	0.52	4.13	0.94	0.72	3.03	0.89	1.10	2.42	0.88
		SNV	33	7	0.53	4.11	0.94	0.79	2.75	0.87	0.83	3.10	0.93
		SD	10	5	0.63	3.44	0.92	0.80	2.70	0.86	1.12	1.94	0.83
		SD+LD	13	4	0.66	3.29	0.91	0.80	2.71	0.86	1.34	1.59	0.78
		SNV+SD	10	5	0.60	3.59	0.92	0.78	2.78	0.87	1.04	2.16	0.87
SR	R	None	8	5	1.07	2.02	0.75	1.30	1.66	0.65	0.82	2.67	0.93

<b>EMCVS</b>		SNV	21	9	0.55	3.97	0.94	0.87	2.50	0.84	0.95	<b>2.20</b>	0.86
		SD	13	6	0.67	3.25	0.91	0.92	2.35	0.82	1.00	<b>2.07</b>	0.87
		SD+LD	17	6	0.74	2.94	0.88	1.04	2.08	0.77	0.88	<b>2.37</b>	0.89
		SNV+SD	20	7	0.60	3.59	0.92	0.88	2.46	0.84	0.91	<b>2.09</b>	0.86
	<b>Log(1/R)</b>	None	8	5	0.90	2.39	0.83	1.12	1.93	0.73	1.07	<b>2.12</b>	0.86
		SNV	16	7	0.52	4.16	0.94	0.82	2.64	0.86	0.81	<b>3.16</b>	0.93
		SD	9	7	0.54	4.05	0.94	0.73	2.96	0.89	1.38	<b>1.81</b>	0.79
		SD+LD	17	4	0.67	3.25	0.91	0.85	2.55	0.85	1.34	<b>1.64</b>	0.79
		SNV+SD	17	4	0.65	3.32	0.91	0.86	2.51	0.84	1.15	<b>1.98</b>	0.85
	<b>R</b>	None	24	5	0.44	4.94	0.96	0.55	3.91	0.93	1.51	<b>1.47</b>	0.77
		SNV	18	4	0.45	4.85	0.96	0.56	3.85	0.93	1.73	<b>1.19</b>	0.73
		SD	13	3	0.75	2.90	0.88	0.87	2.49	0.84	1.00	<b>1.78</b>	0.80
		SD+LD	7	5	0.63	3.43	0.91	0.77	2.80	0.87	1.15	<b>1.60</b>	0.75
<b>EMCVS</b>		SNV+SD	6	3	0.63	3.44	0.92	0.72	3.01	0.89	1.02	<b>1.84</b>	0.79
		None	9	5	0.44	4.93	0.96	0.55	3.93	0.94	1.03	<b>2.30</b>	0.87
	<b>Log(1/R)</b>	SNV	7	6	0.48	4.51	0.95	0.65	3.34	0.91	1.00	<b>2.40</b>	0.88
		SD	10	3	0.66	3.29	0.91	0.75	2.89	0.88	1.04	<b>1.96</b>	0.83
		SD+LD	11	3	0.67	3.23	0.90	0.77	2.83	0.88	1.12	<b>1.87</b>	0.82
		SNV+SD	9	4	0.53	4.08	0.94	0.65	3.36	0.91	0.84	<b>2.50</b>	0.89

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for 10 °C is highlighted in bold.

Appendix 3. Performance of the TVC PLS-R models developed using VIS-SWNIR (445 - 970 nm ) data from beef *LD* samples stored at 4 °C or 10 °C. PLS full spectral range on reflectance (R) and logarithmic transformed ( $\log(1/R)$ ) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre-treatment	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	106	6	0.95	2.46	0.83	1.11	2.11	0.78	1.40	1.52	0.75
		SNV	106	6	0.85	2.76	0.87	1.01	2.32	0.81	1.33	1.47	0.78
		SD	100	5	0.96	2.43	0.83	1.19	1.96	0.74	1.11	1.76	0.79
		SNV+SD	100	7	0.86	2.72	0.87	1.09	2.15	0.78	0.98	1.99	0.84
		SD+LD	100	6	0.92	2.53	0.84	1.15	2.04	0.76	1.22	1.61	0.76
	Log(1/R)	None	106	6	0.92	2.54	0.85	1.17	2.00	0.76	1.23	1.71	0.77
		SNV	106	6	0.83	2.83	0.87	0.98	2.39	0.83	1.36	1.44	0.73
		SD	100	5	0.90	2.59	0.85	1.11	2.11	0.78	1.11	1.80	0.79
		SNV+SD	100	4	0.94	2.50	0.84	1.09	2.14	0.78	1.11	1.76	0.79
		SD+LD	100	4	0.94	2.49	0.84	1.13	2.07	0.77	1.16	1.71	0.77
VIP	R	None	8	5	1.03	2.28	0.81	1.13	2.06	0.77	1.51	1.42	0.69
		SNV	22	7	0.86	2.73	0.87	0.96	2.43	0.83	1.55	1.28	0.74
		SD	7	4	1.19	1.96	0.74	1.29	1.82	0.70	1.08	1.85	0.80
		SNV+SD	8	3	1.21	1.93	0.73	1.32	1.77	0.68	1.31	1.63	0.74
		SD+LD	19	6	1.12	2.09	0.77	1.26	1.85	0.71	1.32	1.51	0.72
	Log(1/R)	None	8	3	1.04	2.26	0.80	1.09	2.14	0.78	1.51	1.37	0.65
		SNV	10	6	0.92	2.55	0.85	1.03	2.28	0.81	1.24	1.68	0.77
		SD	30	4	0.99	2.37	0.82	1.12	2.08	0.77	1.15	1.76	0.78
		SNV+SD	24	5	0.95	2.46	0.83	1.10	2.12	0.78	1.06	1.88	0.80
		SD+LD	15	5	1.03	2.27	0.81	1.15	2.03	0.76	1.28	1.57	0.72
SR	R	None	7	5	1.41	1.66	0.64	1.59	1.47	0.54	1.44	1.60	0.74
		SNV	14	8	1.11	2.11	0.78	1.24	1.89	0.72	1.27	1.74	0.81

EMCVS	Log(1/R)	SD	16	6	1.08	2.16	0.79	1.24	1.89	0.72	1.17	1.70	0.78
		SNV+SD	11	6	1.10	2.13	0.78	1.25	1.87	0.72	1.25	1.58	0.74
		SD+LD	17	6	1.02	2.29	0.81	1.20	1.96	0.74	1.24	1.60	0.75
		None	14	7	1.02	2.30	0.81	1.23	1.90	0.73	1.27	1.63	0.74
		SNV	17	5	1.02	2.29	0.81	1.22	1.92	0.73	1.24	1.61	0.75
		SD	13	7	0.97	2.40	0.83	1.15	2.03	0.76	1.23	1.70	0.76
		SNV+SD	17	5	1.07	2.18	0.79	1.24	1.89	0.72	1.41	1.43	0.68
		SD+LD	14	6	0.99	2.37	0.82	1.16	2.02	0.76	1.11	1.81	0.79
	R	None	3	2	1.02	1.65	0.63	1.07	1.57	0.59	1.66	1.29	0.60
		SNV	9	6	0.81	2.90	0.88	0.90	2.61	0.85	1.56	1.27	0.74
		SD	3	3	1.10	2.13	0.78	1.17	2.01	0.75	1.01	1.97	0.83
		SNV+SD	8	4	0.94	2.48	0.84	1.05	2.22	0.80	0.95	2.10	0.85
		SD+LD	4	3	0.92	2.55	0.85	0.96	2.43	0.83	1.24	1.61	0.76
		None	23	13	0.61	3.82	0.93	0.77	3.04	0.89	0.88	2.25	0.86
		SNV	15	6	0.91	2.57	0.85	1.01	2.32	0.81	1.20	1.71	0.78
		Log(1/R)	SD	8	6	0.93	2.50	0.84	1.05	2.22	0.80	1.21	1.69
	SNV+SD	8	3	0.95	2.45	0.83	1.01	2.31	0.81	0.96	2.10	0.84	
	SD+LD	9	3	0.95	2.45	0.83	1.03	2.27	0.81	1.04	1.92	0.81	

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for 10 °C is highlighted in bold.



Appendix 4. Performance of the TVC PLS-R models developed using NIR (957 - 1664 nm ) data from beef *LD* samples stored at 4 °C, PLS full spectral range on reflectance (R) and logarithmic transformed (log(1/R)) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral Pre-treatment	#	#	Calibration			Cross validation			Prediction			
		Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	102	8	0.66	3.58	0.92	1.14	2.07	0.78	2.75	0.94	0.45
		SD	96	5	0.84	2.79	0.87	1.17	2.00	0.75	1.97	1.68	0.84
		SD+LD	96	5	0.84	2.79	0.87	1.15	2.04	0.76	1.91	1.72	0.84
		SNV+SD	96	5	0.87	2.70	0.86	1.19	1.97	0.74	1.66	1.69	0.83
	Log(1/R)	None	102	6	0.82	2.87	0.88	1.21	1.94	0.75	3.50	0.69	0.08
		SD	96	5	0.92	2.55	0.85	1.45	1.62	0.63	0.87	2.23	0.91
		SD+LD	96	5	0.93	2.52	0.84	1.43	1.64	0.64	0.83	2.33	0.91
		SNV+SD	96	4	0.72	3.28	0.91	0.97	2.42	0.83	1.19	1.63	0.82
VIP	R	None	12	7	0.66	3.55	0.92	1.03	2.28	0.82	2.89	1.33	0.61
		SD	17	5	0.85	2.76	0.87	1.14	2.06	0.77	2.74	1.23	0.57
		SD+LD	17	5	0.93	2.52	0.84	1.20	1.95	0.74	1.91	1.59	0.72
		SNV+SD	12	7	0.83	2.82	0.87	1.08	2.17	0.79	1.94	1.45	0.68
	Log(1/R)	None	14	7	0.60	3.93	0.94	0.86	2.72	0.87	2.88	0.96	0.27
		SD	11	6	0.86	2.72	0.86	1.11	2.11	0.78	1.58	2.25	0.86
		SD+LD	8	6	0.88	2.68	0.86	1.08	2.17	0.79	1.22	2.07	0.86
		SNV+SD	7	4	0.76	3.07	0.89	0.94	2.50	0.84	1.24	1.87	0.80
SR	R	None	42	6	1.02	2.29	0.81	1.32	1.78	0.69	1.57	1.78	0.86
		SD	4	3	1.21	1.94	0.73	1.38	1.70	0.66	1.22	1.91	0.82
		SD+LD	2	1	1.57	1.49	0.55	1.64	1.43	0.51	1.73	1.37	0.64
		SNV+SD	1	1	1.51	1.55	0.58	1.59	1.48	0.54	2.28	1.01	0.32
	Log(1/R)	None	29	6	1.04	2.26	0.80	1.40	1.68	0.66	1.12	2.26	0.87
		SD	6	2	1.44	1.63	0.62	1.58	1.49	0.55	1.23	2.00	0.90

		SD+LD	4	4	1.35	1.74	0.67	1.60	1.47	0.54	1.13	2.12	0.91
		SNV+SD	1	1	1.67	1.40	0.49	1.75	1.34	0.44	2.56	0.90	0.15
EMCVS	R	None	8	5	0.72	3.24	0.90	1.00	2.34	0.82	3.91	0.82	0.07
		SD	16	4	0.94	2.50	0.84	1.14	2.06	0.76	2.83	1.30	0.63
		SD+LD	6	4	0.93	2.52	0.84	1.10	2.14	0.78	1.98	1.59	0.73
		SNV+SD	8	6	0.65	3.63	0.92	0.79	2.95	0.89	1.15	2.18	0.85
		None	19	5	0.86	2.69	0.86	1.06	2.20	0.79	3.85	0.75	0.01
	Log(1/R)	SD	10	4	0.80	2.93	0.88	1.01	2.32	0.82	0.93	2.48	0.89
		<b>SD+LD</b>	<b>13</b>	<b>4</b>	<b>0.78</b>	<b>3.03</b>	<b>0.89</b>	<b>1.01</b>	<b>2.31</b>	<b>0.81</b>	<b>0.92</b>	<b>3.00</b>	<b>0.92</b>
		SNV+SD	5	4	0.73	3.22	0.90	0.88	2.68	0.86	1.51	1.54	0.70

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for 4 °C is highlighted in bold.

Appendix 5. Performance of the TVC PLS-R models developed using NIR (957 - 1664 nm ) data from beef *LD* samples stored at 10 °C, PLS full spectral range on reflectance (R) and logarithmic transformed (log(1/R)) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Pre-treatment	#	#	Calibration			Cross validation			Prediction			
		Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	102	11	0.59	3.68	0.93	1.06	2.04	0.77	1.90	1.16	0.62
		SNV	102	5	1.03	2.10	0.77	1.39	1.55	0.60	2.19	1.23	0.54
		SD	96	7	0.71	3.07	0.89	1.21	1.79	0.70	2.05	0.98	0.47
		SD+LD	96	4	1.27	1.71	0.66	1.52	1.43	0.51	1.67	1.28	0.55
		SNV+SD	96	6	0.73	2.97	0.89	1.07	2.02	0.76	1.59	1.31	0.63
	Log(1/R)	None	102	11	0.55	3.91	0.93	1.07	2.02	0.76	2.38	0.80	0.35
		SNV	102	12	0.41	5.29	0.96	0.90	2.41	0.83	1.67	1.53	0.73
		SD	96	7	0.68	3.18	0.90	1.11	1.95	0.74	1.38	1.33	0.64
		SD+LD	96	6	0.79	2.75	0.87	1.29	1.68	0.65	1.37	1.31	0.63
		SNV+SD	96	7	0.61	3.56	0.92	1.03	2.12	0.78	1.19	1.57	0.72
EMCVS	R	None	19	9	0.68	3.19	0.90	1.00	2.17	0.79	1.79	1.02	0.51
		SNV	4	4	1.00	2.10	0.77	1.17	1.79	0.69	1.93	1.05	0.41
		SD	8	4	0.90	2.40	0.83	1.04	2.08	0.77	1.59	1.34	0.64
		SD+LD	7	4	0.81	2.67	0.86	0.96	2.26	0.81	1.37	1.31	0.65
		SNV+SD	14	3	0.80	2.72	0.86	0.90	2.39	0.83	1.64	1.43	0.69
	Log(1/R)	None	12	8	0.63	3.46	0.92	0.88	2.45	0.84	2.57	0.70	0.31
		SNV	19	9	0.44	4.93	0.96	0.67	3.22	0.90	1.15	2.23	0.87
		SD	12	5	0.78	2.78	0.87	0.96	2.25	0.80	1.71	1.35	0.67
		SD+LD	9	4	1.03	2.11	0.78	1.14	1.91	0.73	1.49	1.15	0.51
		SNV+SD	15	5	0.71	3.05	0.89	0.92	2.36	0.82	1.45	1.30	0.60
SR	R	None	1	1	2.14	1.01	0.03	2.32	0.93	0.12	1.93	0.90	0.29
		SNV	7	5	1.28	1.69	0.65	1.58	1.37	0.49	2.22	0.84	0.26

	<b>Log(1/R)</b>	SD	2	1	1.45	1.49	0.55	1.55	1.40	0.49	1.80	<b>0.96</b>	0.30
		SD+LD	1	1	1.72	1.26	0.37	1.84	1.18	0.29	2.26	<b>0.77</b>	0.12
		SNV+SD	1	1	1.91	1.14	0.23	2.03	1.07	0.14	1.90	<b>1.04</b>	0.36
		None	1	1	2.14	1.01	0.02	2.32	0.93	0.15	1.95	<b>0.89</b>	0.26
		SNV	1	1	1.92	1.13	0.21	2.04	1.06	0.13	1.50	<b>1.23</b>	0.72
		SD	1	1	1.91	1.13	0.22	2.00	1.08	0.15	1.74	<b>0.99</b>	0.29
		SD+LD	1	1	1.91	1.14	0.23	2.00	1.09	0.16	1.73	<b>1.00</b>	0.30
		SNV+SD	4	3	1.49	1.46	0.53	1.78	1.22	0.36	1.82	<b>1.07</b>	0.39
		None	6	4	1.32	1.64	0.63	1.55	1.40	0.49	2.17	<b>0.89</b>	0.28
		SNV	21	8	0.74	2.92	0.88	1.14	1.90	0.74	1.95	<b>1.30</b>	0.60
<b>VIP</b>	<b>R</b>	SD	6	4	1.07	2.02	0.76	1.22	1.78	0.69	1.47	<b>1.64</b>	0.76
		SD+LD	5	3	1.40	1.55	0.58	1.59	1.36	0.47	1.91	<b>1.14</b>	0.54
		SNV+SD	9	4	0.87	2.48	0.84	1.03	2.10	0.77	1.82	<b>1.71</b>	0.77
		None	13	9	0.82	2.65	0.86	1.18	1.84	0.71	1.78	<b>1.79</b>	0.86
		SNV	20	10	0.54	4.00	0.94	1.04	2.07	0.78	1.82	<b>1.12</b>	0.53
	<b>Log(1/R)</b>	SD	24	8	0.67	3.23	0.90	0.98	2.22	0.80	1.40	<b>1.35</b>	0.66
		SD+LD	22	6	0.90	2.42	0.83	1.18	1.84	0.70	1.61	<b>1.15</b>	0.54
		SNV+SD	31	8	0.67	3.23	0.90	0.98	2.21	0.80	1.14	<b>1.65</b>	0.75
		None	6	4	1.32	1.64	0.63	1.55	1.40	0.49	2.17	<b>0.89</b>	0.28
		SNV	21	8	0.74	2.92	0.88	1.14	1.90	0.74	1.95	<b>1.30</b>	0.60

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for 10 °C is highlighted in bold.

Appendix 6. Performance of the TVC PLS-R models developed using NIR (957 - 1664 nm ) data from beef *LD* samples stored either 4 °C or 10 °C. PLS full spectral range on reflectance (R) and logarithmic transformed (log(1/R)) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre- treatment	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	102	6	1.36	1.72	0.66	1.57	1.49	0.55	2.19	0.89	0.29
		SNV	102	13	0.86	2.72	0.86	1.27	1.85	0.71	2.58	1.00	0.44
		SD	96	6	1.19	1.97	0.74	1.40	1.67	0.64	1.27	1.53	0.71
		SNV+SD	96	7	1.06	2.20	0.79	1.36	1.72	0.67	1.58	1.25	0.61
		SD+LD	96	6	1.20	1.94	0.73	1.42	1.65	0.64	1.29	1.52	0.71
	Log(1/R)	None	102	5	1.51	1.55	0.58	1.74	1.34	0.45	2.78	0.72	0.14
		SNV	102	13	0.84	2.79	0.87	1.38	1.70	0.67	1.44	1.35	0.64
		SD	96	6	1.18	1.98	0.74	1.47	1.59	0.61	1.21	1.62	0.74
		SNV+SD	96	6	1.15	2.03	0.76	1.48	1.58	0.61	1.29	1.51	0.70
		SD+LD	96	6	1.17	2.01	0.75	1.47	1.59	0.61	1.18	1.66	0.76
VIP	R	None	9	5	1.41	1.65	0.63	1.56	1.50	0.56	2.52	0.79	0.22
		SNV	20	13	0.92	2.53	0.84	1.19	1.97	0.75	1.45	1.42	0.68
		SD	27	6	1.18	1.99	0.75	1.37	1.70	0.66	1.41	1.40	0.65
		SNV+SD	17	6	1.17	2.01	0.75	1.35	1.73	0.67	1.41	1.41	0.65
		SD+LD	6	4	1.36	1.72	0.66	1.47	1.59	0.60	1.34	1.46	0.67
	Log(1/R)	None	32	10	1.24	1.89	0.72	1.52	1.54	0.59	2.33	0.84	0.27
		SNV	10	5	1.35	1.73	0.67	1.52	1.53	0.58	1.93	1.09	0.44
		SD	17	6	1.23	1.90	0.72	1.41	1.66	0.64	1.29	1.58	0.72
		SNV+SD	12	6	1.21	1.93	0.73	1.40	1.67	0.65	1.17	1.66	0.75
		SD+LD	17	6	1.27	1.83	0.70	1.43	1.63	0.63	1.30	1.51	0.70
SR	R	None	28	7	1.40	1.67	0.64	1.93	1.21	0.41	1.31	1.49	0.70
		SNV	1	1	1.92	1.22	0.33	1.98	1.18	0.28	2.19	0.89	0.13

EMCVS	Log(1/R)	SD	1	1	1.77	1.32	0.43	1.81	1.29	0.40	1.88	1.05	0.39
		SNV+SD	5	4	1.49	1.57	0.59	1.62	1.44	0.52	1.58	1.24	0.55
		SD+LD	1	1	1.72	1.36	0.46	1.77	1.32	0.43	1.93	1.02	0.37
		None	27	7	1.44	1.63	0.62	1.84	1.27	0.42	1.48	1.33	0.63
		SNV	11	3	1.91	1.23	0.34	2.07	1.13	0.23	2.32	0.86	0.11
		SD	1	1	1.94	1.20	0.31	1.98	1.18	0.28	1.84	1.13	0.45
		SNV+SD	1	1	2.10	1.12	0.20	2.16	1.08	0.15	2.14	0.92	0.18
		SD+LD	1	1	1.93	1.21	0.32	1.97	1.19	0.29	1.83	1.14	0.46
	R	None	6	4	1.42	1.65	0.63	1.55	1.52	0.57	2.12	1.00	0.37
		SNV	19	11	0.85	2.76	0.87	1.09	2.15	0.79	1.77	1.23	0.56
		SD	10	4	1.31	1.78	0.69	1.42	1.65	0.63	1.48	1.45	0.67
		SNV+SD	12	5	1.04	2.24	0.80	1.18	1.98	0.74	1.15	1.75	0.77
		SD+LD	11	4	1.32	1.77	0.68	1.43	1.63	0.63	1.55	1.28	0.59
		None	12	6	1.36	1.70	0.65	1.55	1.49	0.55	2.08	0.95	0.32
		<b>SNV</b>	<b>19</b>	<b>11</b>	<b>0.90</b>	<b>2.60</b>	<b>0.85</b>	<b>1.14</b>	<b>2.05</b>	<b>0.77</b>	<b>1.03</b>	<b>1.92</b>	<b>0.81</b>
		Log(1/R) SD	5	4	1.29	1.81	0.70	1.41	1.66	0.64	1.46	1.35	0.63
	Log(1/R)	SNV+SD	5	3	1.17	1.96	0.74	1.28	1.80	0.69	1.63	1.20	0.53
		SD+LD	6	4	1.25	1.87	0.72	1.37	1.71	0.66	1.48	1.35	0.64

Appendix 7. Performance of the best TVC PLS-R models developed using the LL data fusion of VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data from beef LD samples stored at 4 °C. PLS full spectral range on reflectance (R) and logarithmic transformed ( $\log(1/R)$ ) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre-treatment	Bands	LV	RMSEcv	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	208	7	0.87	2.85	0.88	1.53	1.63	0.66	1.73	1.23	0.68
		SNV	208	7	0.81	3.06	0.89	1.34	1.86	0.73	1.69	1.30	0.67
		SD	202	6	0.84	2.97	0.89	1.35	1.86	0.71	1.18	1.75	0.86
		SD+LD	202	6	0.85	2.94	0.88	1.38	1.81	0.70	1.17	1.76	0.86
		SNV+SD	202	6	0.82	3.05	0.89	1.15	2.17	0.79	1.35	1.52	0.77
	Log(1/R)	None	198	6	0.75	3.30	0.91	1.45	1.74	0.67	1.42	1.51	0.78
		SNV	208	6	0.75	3.32	0.91	1.06	2.35	0.82	1.36	1.57	0.75
		SD	208	7	0.71	3.49	0.92	1.11	2.25	0.80	1.32	1.66	0.78
		SD+LD	208	9	0.71	3.51	0.92	1.28	1.95	0.75	1.38	1.86	0.85
		SNV+SD	202	6	0.72	3.44	0.92	1.17	2.13	0.78	1.25	1.66	0.83
VIP	R	None	4	3	1.11	2.25	0.80	1.23	2.03	0.76	1.62	1.45	0.71
		SNV	24	5	0.97	2.56	0.85	1.26	1.97	0.74	1.74	1.27	0.63
		SD	22	11	0.56	4.42	0.95	1.11	2.27	0.81	1.74	1.65	0.83
		SD+LD	22	8	0.76	3.28	0.91	1.09	2.30	0.81	1.16	1.90	0.88
		SNV+SD	32	6	0.90	2.78	0.87	1.23	2.03	0.76	1.22	1.87	0.82
	Log(1/R)	None	10	7	0.88	2.82	0.87	1.31	1.91	0.75	1.48	1.52	0.72
		SNV	18	12	0.52	4.82	0.96	0.97	2.56	0.87	1.51	1.45	0.75
		SD	35	9	0.57	4.35	0.95	1.00	2.50	0.84	1.45	1.56	0.75
		SD+LD	29	12	0.50	4.99	0.96	0.95	2.64	0.86	1.58	1.61	0.74
		SNV+SD	10	7	0.86	2.90	0.88	1.32	1.90	0.72	1.34	1.71	0.77
SR	R	None	15	7	1.07	2.34	0.82	1.56	1.60	0.66	1.53	1.62	0.79

<b>Log(1/R)</b>	SNV	17	9	0.92	2.72	0.87	1.36	1.83	0.71	1.00	<b>2.22</b>	0.87
	SD	8	4	1.00	2.49	0.84	1.27	1.96	0.74	1.60	<b>1.37</b>	0.68
	SD+LD	8	3	1.11	2.25	0.80	1.29	1.94	0.73	1.72	<b>1.28</b>	0.66
	SNV+SD	2	1	1.35	1.85	0.71	1.45	1.72	0.66	1.77	<b>1.29</b>	0.66
	None	15	8	0.91	2.73	0.87	1.39	1.80	0.73	1.52	<b>1.48</b>	0.74
	SNV	21	6	1.14	2.19	0.79	1.58	1.59	0.62	1.41	<b>1.58</b>	0.77
	SD	7	6	0.85	2.94	0.88	1.09	2.29	0.81	2.64	<b>1.35</b>	0.64
	SD+LD	1	1	1.24	2.01	0.75	1.31	1.90	0.72	2.08	<b>1.05</b>	0.44
	SNV+SD	8	5	0.91	2.72	0.87	1.13	2.21	0.80	1.97	<b>1.78</b>	0.83
	None	9	7	0.97	2.58	0.85	1.28	1.95	0.75	1.33	<b>1.66</b>	0.76
<b>EMCVS</b>	SNV	5	4	1.02	2.32	0.81	1.15	2.06	0.76	1.74	<b>1.29</b>	0.58
	SD	9	6	0.71	3.51	0.92	0.91	2.74	0.87	1.71	<b>1.29</b>	0.81
	SD+LD	10	6	0.74	3.36	0.91	0.94	2.64	0.86	1.69	<b>1.36</b>	0.76
	SNV+SD	28	4	0.92	2.72	0.86	1.08	2.30	0.81	1.28	<b>1.81</b>	0.82
	None	25	11	0.53	4.73	0.96	0.93	2.68	0.87	3.12	<b>1.30</b>	0.60
	SNV	11	4	0.70	3.54	0.92	0.94	2.66	0.86	1.94	<b>1.34</b>	0.61
	SD	6	4	0.77	3.22	0.90	0.92	2.72	0.87	1.54	<b>1.43</b>	0.68
	SD+LD	5	4	0.85	2.93	0.88	0.98	2.54	0.85	1.33	<b>1.66</b>	0.80
	SNV+SD	8	5	0.75	3.32	0.91	0.92	2.72	0.87	1.30	<b>1.71</b>	0.77
	None	9	7	0.97	2.58	0.85	1.28	1.95	0.75	1.33	<b>1.66</b>	0.76

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for the LL data fusion of 4 °C data is highlighted in bold.



Appendix 8. Performance of the best TVC PLS-R models developed using the LL data fusion of VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data from beef LD samples stored at 10 °C. PLS full spectral range on reflectance (R) and logarithmic transformed ( $\log(1/R)$ ) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Chemometric method	Spectral	#	#	Calibration			Cross validation			Prediction			
	Pre-treatment	Bands	LV	RMSEcv	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	208	8	0.43	5.04	0.96	0.76	2.87	0.88	1.39	1.61	0.73
		SNV	208	7	0.42	5.20	0.96	0.67	3.25	0.91	1.41	1.87	0.79
		SD	202	7	0.58	3.72	0.93	0.95	2.28	0.81	1.11	1.86	0.79
		SD+LD	202	7	0.58	3.73	0.93	0.95	2.29	0.81	1.10	1.87	0.79
		SNV+SD	202	4	0.70	3.08	0.89	0.93	2.32	0.82	0.88	2.46	0.89
	Log(1/R)	None	208	8	0.44	4.90	0.96	0.77	2.82	0.88	1.64	1.59	0.76
		SNV	208	6	0.55	3.95	0.94	0.83	2.61	0.85	1.33	1.97	0.81
		SD	202	6	0.54	4.02	0.94	0.84	2.59	0.85	1.41	1.75	0.79
		SD+LD	202	6	0.54	4.05	0.94	0.83	2.61	0.85	1.39	1.78	0.80
		SNV+SD	202	6	0.48	4.49	0.95	0.76	2.85	0.88	1.23	1.97	0.83
EMCVS	R	None	20	6	0.43	5.09	0.96	0.59	3.68	0.93	1.26	1.89	0.81
		SNV	128	6	0.42	5.11	0.96	0.61	3.54	0.92	1.30	1.92	0.82
		SD	9	6	0.52	4.16	0.94	0.65	3.36	0.91	0.86	2.09	0.84
		SD+LD	10	7	0.49	4.45	0.95	0.64	3.40	0.91	0.97	1.98	0.84
		SNV+SD	12	5	0.49	4.39	0.95	0.68	3.17	0.90	1.20	1.76	0.77
	Log(1/R)	None	11	6	0.46	4.66	0.95	0.58	3.71	0.93	0.94	3.03	0.94
		SNV	7	5	0.59	3.69	0.93	0.75	2.90	0.88	1.13	2.22	0.86
		SD	7	3	0.62	3.49	0.92	0.71	3.04	0.89	0.98	2.05	0.84
		SD+LD	20	3	0.63	3.41	0.91	0.73	2.96	0.89	1.11	1.87	0.82
		SNV+SD	9	4	0.55	3.94	0.94	0.66	3.30	0.91	1.03	2.12	0.85
SR	R	None	8	5	1.07	2.02	0.75	1.30	1.66	0.65	0.82	2.67	0.93
		SNV	20	8	0.65	3.32	0.91	0.98	2.21	0.80	0.89	2.56	0.91

	<b>Log(1/R)</b>	SD	13	6	0.67	3.25	0.91	0.92	2.35	0.82	1.00	<b>2.07</b>	0.87
		SD+LD	23	7	0.74	2.93	0.88	1.08	2.01	0.75	0.74	<b>2.51</b>	0.90
		SNV+SD	11	6	0.73	2.96	0.89	0.97	2.24	0.80	1.20	<b>1.81</b>	0.84
		None	8	5	0.90	2.39	0.83	1.12	1.93	0.73	1.07	<b>2.12</b>	0.86
		SNV	25	8	0.70	3.08	0.89	1.14	1.91	0.73	1.50	<b>1.40</b>	0.84
		SD	25	5	0.54	4.04	0.94	0.80	2.70	0.86	1.13	<b>2.02</b>	0.83
		SD+LD	10	8	0.53	4.11	0.94	0.80	2.71	0.86	1.20	<b>1.86</b>	0.80
		SNV+SD	24	5	0.55	3.95	0.94	0.77	2.82	0.87	1.02	<b>2.20</b>	0.86
<b>VIP</b>	<b>R</b>	None	21	10	0.40	5.48	0.97	0.59	3.68	0.93	1.21	<b>1.77</b>	0.78
		SNV	27	5	0.48	4.48	0.95	0.68	3.20	0.90	1.04	<b>2.15</b>	0.86
		SD	20	8	0.60	3.62	0.92	0.88	2.47	0.84	1.21	<b>1.72</b>	0.77
		SD+LD	16	8	0.61	3.56	0.92	0.89	2.43	0.83	1.24	<b>1.66</b>	0.75
		SNV+SD	14	5	0.73	2.97	0.89	0.94	2.31	0.81	0.84	<b>2.50</b>	0.89
	<b>Log(1/R)</b>	None	14	7	0.48	4.52	0.95	0.68	3.17	0.90	1.11	<b>2.35</b>	0.87
		SNV	7	5	0.61	3.57	0.92	0.75	2.88	0.88	1.10	<b>2.42</b>	0.88
		SD	10	5	0.63	3.44	0.92	0.80	2.70	0.86	1.12	<b>1.94</b>	0.83
		SD+LD	14	4	0.63	3.43	0.92	0.80	2.70	0.86	1.16	<b>1.85</b>	0.82
		SNV+SD	10	5	0.58	3.72	0.93	0.74	2.92	0.88	1.01	<b>2.12</b>	0.85

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for the LLDF of 10 °C data is highlighted in bold.

Appendix 9. Performance of the best TVC PLS-R models developed using the LL data fusion of VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data from beef *LD* samples stored at either 4 °C or 10 °C. PLS full spectral range on reflectance (R) and logarithmic transformed ( $\log(1/R)$ ) spectral data is compared with spectral pre-treatments (SNV, SD, SD+LD, SNV+SD) and band selection methods (VIP, SR and EMCVS).

Regression Model	Pre-treatment	# Bands	# LV	Calibration			Cross validation			Prediction			
				RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p	
PLS	R	None	208	8	0.86	2.72	0.86	1.06	2.21	0.8	1.38	1.43	0.72
		SNV	208	7	0.79	2.95	0.89	1.01	2.32	0.82	1.42	1.38	0.70
		SD	202	6	0.96	2.45	0.83	1.20	1.95	0.74	0.95	2.08	0.85
		SD+LD	202	7	0.89	2.64	0.86	1.16	2.02	0.75	1.00	2.04	0.85
		SNV+SD	202	7	0.83	2.82	0.87	1.07	2.19	0.79	1.12	1.86	0.81
	Log(1/R)	None	208	8	0.83	2.81	0.87	1.12	2.09	0.78	1.44	1.36	0.66
		SNV	208	5	0.94	2.50	0.84	1.09	2.14	0.78	1.33	1.49	0.71
		SD	202	6	0.88	2.66	0.86	1.10	2.13	0.78	1.17	1.71	0.77
		SD+LD	202	6	0.89	2.63	0.86	1.11	2.11	0.78	1.15	1.76	0.78
		SNV+SD	202	5	0.93	2.52	0.84	1.14	2.06	0.76	1.18	1.75	0.78
VIP	R	None	30	8	0.96	2.44	0.83	1.09	2.15	0.78	1.48	1.43	0.69
		SNV	13	7	0.96	2.45	0.83	1.09	2.15	0.78	1.40	1.44	0.73
		SD	15	7	1.03	2.27	0.81	1.22	1.91	0.73	1.07	1.86	0.80
		SD+LD	11	6	1.02	2.30	0.81	1.18	1.98	0.75	1.15	1.73	0.79
		SNV+SD	29	10	0.82	2.84	0.88	1.10	2.13	0.78	1.09	1.83	0.81
	Log(1/R)	None	10	4	1.06	2.21	0.8	1.14	2.06	0.76	1.49	1.35	0.66
		SNV	22	6	0.92	2.55	0.85	1.04	2.24	0.8	1.31	1.53	0.73
		SD	6	4	1.10	2.12	0.78	1.19	1.97	0.74	1.18	1.74	0.77
		SD+LD	6	3	1.08	2.17	0.79	1.12	2.10	0.77	1.21	1.67	0.75
		SNV+SD	10	4	1.04	2.25	0.8	1.17	2.00	0.75	1.27	1.66	0.75
SR	R	None	7	5	1.41	1.66	0.64	1.59	1.47	0.54	1.44	1.60	0.74

	SNV	20	9	0.97	2.42	0.83	1.33	1.75	0.68	1.24	1.75	0.78
	SD	12	8	1.05	2.23	0.80	1.23	1.91	0.73	1.06	1.87	0.82
	SD+LD	24	6	1.07	2.19	0.79	1.23	1.90	0.72	1.24	1.61	0.75
	SNV+SD	11	6	1.13	2.06	0.76	1.32	1.78	0.69	1.33	1.50	0.71
	None	14	7	1.02	2.3	0.81	1.23	1.90	0.73	1.27	1.63	0.74
Log(1/R)	SNV	18	8	1.09	2.15	0.78	1.31	1.79	0.69	1.19	1.96	0.82
	SD	13	7	0.97	2.4	0.83	1.15	2.03	0.76	1.23	1.70	0.76
	SD+LD	19	5	0.98	2.38	0.82	1.1	2.12	0.78	1.25	1.61	0.74
	SNV+SD	15	7	0.95	2.46	0.84	1.15	2.04	0.76	1.36	1.59	0.72
EMCVS	None	15	5	0.93	2.51	0.84	1.07	2.18	0.79	1.32	1.63	0.77
	SNV	52	6	0.86	2.73	0.87	0.98	2.4	0.83	1.37	1.48	0.73
	SD	6	3	1.06	2.21	0.80	1.13	2.06	0.76	1.15	1.73	0.79
	SD+LD	<b>35</b>	<b>4</b>	<b>0.96</b>	<b>2.45</b>	<b>0.83</b>	<b>1.12</b>	<b>2.08</b>	<b>0.77</b>	<b>0.87</b>	<b>2.27</b>	<b>0.88</b>
	SNV+SD	15	4	0.90	2.61	0.85	0.99	2.36	0.82	1.03	2.10	0.85
	None	7	5	0.97	2.40	0.83	1.06	2.21	0.8	1.58	1.37	0.65
	SNV	17	8	0.87	2.68	0.86	1.03	2.26	0.81	1.32	1.70	0.76
	SD	6	3	0.99	2.36	0.82	1.07	2.18	0.79	1.04	1.91	0.81
	SD+LD	5	5	0.93	2.51	0.84	1.01	2.31	0.81	0.98	2.09	0.84
	SNV+SD	3	2	1.04	2.18	0.79	1.08	2.10	0.77	1.24	1.60	0.73

SR, selectivity ratio; VIP, variable importance projection; EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; LD, linear detrend; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for the LLDF of 4 and 10 °C combine data is highlighted in bold.

1 Table 1. TVC of beef *LD* samples (log CFU/g) stored at 4 and 10 °C. Average value  $\pm$  standard deviation (n = 3). Superscripts show the  
 2 statistical significance between samples obtained with Tukey-Kramer test at  $\alpha = 0.05$ .

Temperature	Time (hours)	Sample id. /TVC					
		S1	S2	S3	S7	S8	S9
4 °C	0	7.1 $\pm$ 0.4 <sup>a,c</sup>	6.9 $\pm$ 0.2 <sup>a,c</sup>	7.0 $\pm$ 0.5 <sup>a,b</sup>	5.3 $\pm$ 0.1 <sup>a</sup>	5.3 $\pm$ 0.4 <sup>a</sup>	3.4 $\pm$ 0.5 <sup>a</sup>
	24	7.1 $\pm$ 0.3 <sup>a,c</sup>	6.9 $\pm$ 0.4 <sup>a,c</sup>	7.2 $\pm$ 0.6 <sup>a,b</sup>	5.3 $\pm$ 0.2 <sup>a</sup>	5.1 $\pm$ 0.4 <sup>a</sup>	3.3 $\pm$ 0.0 <sup>a</sup>
	96	8.9 $\pm$ 0.1 <sup>b,c,d,e</sup>	8.6 $\pm$ 0.2 <sup>b,c,d,f,g</sup>	8.3 $\pm$ 0.3 <sup>a,b,c</sup>	6.6 $\pm$ 0.4 <sup>b</sup>	7.1 $\pm$ 0.7 <sup>b</sup>	4.1 $\pm$ 0.3 <sup>a</sup>
	120	8.1 $\pm$ 0.0 <sup>a,b,c,e</sup>	7.7 $\pm$ 0.1 <sup>a,b,c,d,g</sup>	8.1 $\pm$ 0.6 <sup>a,b,c</sup>	8.7 $\pm$ 0.6 <sup>c</sup>	8.3 $\pm$ 0.2 <sup>c</sup>	5.6 $\pm$ 0.0 <sup>b</sup>
	192	8.8 $\pm$ 0.4 <sup>b,c,d,e</sup>	9.1 $\pm$ 0.2 <sup>b,c,d,e,f,g</sup>	8.4 $\pm$ 0.3 <sup>a,b,c</sup>	9.4 $\pm$ 0.1 <sup>c</sup>	9.4 $\pm$ 0.2 <sup>c</sup>	7.7 $\pm$ 0.5 <sup>c,d</sup>
	264	10.1 $\pm$ 0.7 <sup>b,d,e,f</sup>	10.5 $\pm$ 0.7 <sup>d,e,f</sup>	9.0 $\pm$ 0.1 <sup>b,c,d</sup>	10.9 $\pm$ 0.8 <sup>d</sup>	12.2 $\pm$ 0.1 <sup>d,e</sup>	8.9 $\pm$ 0.1 <sup>c,d,e</sup>
	288	9.5 $\pm$ 0.3 <sup>b,c,d,e,f</sup>	9.7 $\pm$ 0.6 <sup>b,d,e,f,g,h</sup>	10.2 $\pm$ 0.3 <sup>c,d,e</sup>	12.2 $\pm$ 0.3 <sup>e,f</sup>	12.1 $\pm$ 0.3 <sup>d,e,f</sup>	9.2 $\pm$ 0.2 <sup>c,d,e</sup>
	312	9.7 $\pm$ 1.2 <sup>b,c,d,e,f</sup>	9.0 $\pm$ 1.0 <sup>b,c,d,f,g,h</sup>	10.6 $\pm$ 0.7 <sup>d,e</sup>	-	-	-
	336	-	-	-	13.2 $\pm$ 0.2 <sup>e,f,g</sup>	13.5 $\pm$ 0.7 <sup>e,f</sup>	10.5 $\pm$ 1.3 <sup>d,e</sup>
	360	10.6 $\pm$ 0.5 <sup>d,e,f</sup>	11.1 $\pm$ 0.3 <sup>f,h</sup>	11.4 $\pm$ 1.0 <sup>d,e</sup>	14. $\pm$ 0.3 <sup>f,g</sup>	14.1 $\pm$ 0.1 <sup>f,g</sup>	-
Temperature	Time (hours)	Sample id. /TVC					
		S4	S5	S6	S7	S8	S9
10 °C	0	6.1 $\pm$ 0.1 <sup>a</sup>	6.7 $\pm$ 0.1 <sup>a</sup>	6.7 $\pm$ 0.3 <sup>a,b</sup>	5.3 $\pm$ 0.1 <sup>a</sup>	5.3 $\pm$ 0.4 <sup>a</sup>	3.4 $\pm$ 0.5 <sup>a</sup>
	12	6.7 $\pm$ 0.3 <sup>b,c</sup>	7.7 $\pm$ 0.2 <sup>b</sup>	6.9 $\pm$ 0.1 <sup>a,b</sup>	-	-	-
	24	6.8 $\pm$ 0.2 <sup>b,c</sup>	7.3 $\pm$ 0.1 <sup>b</sup>	7.1 $\pm$ 0.1 <sup>a,b,c</sup>	6.0 $\pm$ 0.3 <sup>a</sup>	6.1 $\pm$ 0.8 <sup>a</sup>	3.4 $\pm$ 0.5 <sup>a</sup>
	36	6.9 $\pm$ 4.0 <sup>b,c,d</sup>	7.7 $\pm$ 0.1 <sup>b</sup>	7.1 $\pm$ 0.2 <sup>a,b,c</sup>	-	-	-
	48	7.4 $\pm$ 0.1 <sup>c,d</sup>	7.6 $\pm$ 0.1 <sup>b</sup>	7.5 $\pm$ 0.1 <sup>b,c</sup>	-	-	-
	84	9.2 $\pm$ 0.1 <sup>e,f,h</sup>	9.4 $\pm$ 0.1 <sup>c,d,e,g</sup>	9.3 $\pm$ 0.2 <sup>d,e</sup>	-	-	-
	96	9.4 $\pm$ 0.2 <sup>e,f,h,i</sup>	9.1 $\pm$ 0.2 <sup>c,d</sup>	9.3 $\pm$ 0.1 <sup>d,e</sup>	8.8 $\pm$ 0.2 <sup>b,c</sup>	9.0 $\pm$ 0.1 <sup>b</sup>	7.6 $\pm$ 0.1 <sup>b</sup>
	108	9.9 $\pm$ 0.1 <sup>g,h,i</sup>	9.8 $\pm$ 0.2 <sup>c,e,f,g</sup>	9.6 $\pm$ 0.1 <sup>d,e,f</sup>	-	-	-
	120	9.6 $\pm$ 0.2 <sup>e,f,g,h,i</sup>	9.8 $\pm$ 0.1 <sup>c,e,f,g</sup>	9.8 $\pm$ 0.1 <sup>e,f</sup>	10.5 $\pm$ 0.4 <sup>b,c,d</sup>	10.2 $\pm$ 0.1 <sup>c</sup>	9.3 $\pm$ 0.5 <sup>c</sup>
	132	10.1 $\pm$ 0.2 <sup>g,h,i</sup>	10.0 $\pm$ 0.1 <sup>e,f,g</sup>	9.9 $\pm$ 0.1 <sup>e,f</sup>	-	-	-
	144	9.8 $\pm$ 0.1 <sup>f,g,h,i</sup>	9.9 $\pm$ 0.1 <sup>c,e,f,g</sup>	10.0 $\pm$ 0.2 <sup>e,f</sup>	11.6 $\pm$ 1.4 <sup>c,d</sup>	12.2 $\pm$ 0.2 <sup>d</sup>	11.1 $\pm$ 0.2 <sup>d</sup>
	168	-	-	-	12.3 $\pm$ 0.0 <sup>c,d</sup>	13.1 $\pm$ 0.1 <sup>d</sup>	12.3 $\pm$ 0.3 <sup>e</sup>

3 Values followed by different letters in the same column are significantly different using ANOVA and Tukey test (p < 0.05).

4 Table 2. Performance of the best TVC PLS-R models developed for beef *LD* samples stored at 4 °C using the full spectral range and optimum  
 5 band selection method evaluated for VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data.

Regression model		Pre-treatment		#	#	Calibration			Cross validation			Prediction		
		1 <sup>st</sup>	2 <sup>nd</sup>	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p
<b>445 - 970 nm</b>														
PLS	R	SNV	SD	100	6	0.79	3.15	0.9	1.34	1.86	0.74	1.35	1.61	0.81
EMCVS	log(1/R)	SNV	SD	6	5	0.88	2.83	0.88	1.03	2.42	0.83	1.17	1.88	0.83
<b>957 - 1664 nm</b>														
PLS	log(1/R)	SD	AsLs	96	9	0.42	5.63	0.97	1.03	2.29	0.81	0.99	2.26	0.94
<b>EMCVS</b>	<b>log(1/R)</b>	<b>SD</b>	<b>AsLs</b>	<b>17</b>	<b>7</b>	<b>0.5</b>	<b>4.71</b>	<b>0.95</b>	<b>0.7</b>	<b>3.37</b>	<b>0.91</b>	<b>0.81</b>	<b>3.09</b>	<b>0.95</b>

6 EMCVS, ensemble Monte Carlo variable selection; SD, second derivative; SNV, standard normal variate; AsLs, asymmetric least squares;  
 7 #Bands, wavelengths used for model development; #LVs, latent variables. The best overall model for 4 °C is highlighted in bold.

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Table 3. Performance of the best TVC PLS-R models developed for beef *LD* samples stored at 10 °C using the full spectral range and optimum band selection method evaluated for VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data.

Regression model		Pre-treatment		#	#	Calibration			Cross validation			Prediction		
		1 <sup>st</sup>	2 <sup>nd</sup>	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p
<b>445 - 970 nm</b>														
PLS	R	SD	MS	100	6	0.48	4.52	0.95	0.7	3.08	0.89	1.09	2.84	0.92
EMCVS	R	SD	MS	<b>46</b>	<b>6</b>	<b>0.47</b>	<b>4.60</b>	<b>0.95</b>	<b>0.69</b>	<b>3.16</b>	<b>0.90</b>	<b>0.96</b>	<b>3.32</b>	<b>0.94</b>
<b>957 - 1664 nm</b>														
PLS	log(1/R)	SD	SNV	96	7	0.62	3.49	0.92	1.03	2.11	0.78	1.2	1.59	0.72
EMCVS	log(1/R)	SNV		19	9	0.44	4.93	0.96	0.67	3.22	0.90	1.15	2.23	0.87

EMCVS, ensemble Monte Carlo variable selection; MS, median scaled; SD, second derivative; SNV, standard normal variate; #Bands, wavelengths used for model development; #LVs, latent variables. The best overall model for 10 °C is highlighted in bold.

Table 4. Performance of the best TVC PLS-R models developed for beef *LD* samples stored at either 4 °C or 10 °C using the full spectral range and optimum band selection method evaluated for VIS-SWNIR (445 - 970 nm ) and NIR (957 - 1664 nm) HSI data.

Regression model		Pre-treatment		#	#	Calibration			Cross validation			Prediction		
		1 <sup>st</sup>	2 <sup>nd</sup>	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p
<b>445 - 970 nm</b>														
PLS	R	SNV	SD	100	7	0.86	2.72	0.87	1.09	2.15	0.78	0.98	1.99	0.84
<b>EMCVS</b>	<b>R</b>	<b>SNV</b>	<b>SD</b>	<b>8</b>	<b>4</b>	<b>0.94</b>	<b>2.48</b>	<b>0.84</b>	<b>1.05</b>	<b>2.22</b>	<b>0.80</b>	<b>0.95</b>	<b>2.10</b>	<b>0.85</b>
<b>957 - 1664 nm</b>														
PLS	log(1/R)	SD	LD	202	4	1.17	2.01	0.75	1.47	1.59	0.61	1.18	1.66	0.76
EMCVS	R	SNV	SD	96	6	1.04	2.24	0.80	1.18	1.98	0.74	1.15	1.75	0.77

EMCVS, ensemble Monte Carlo variable selection; SNV, standard normal variate; LD, linear detrend; SD, second derivative; #Bands, wavelengths used for model development; #LVs, latent variables. The overall best model for the combined data of both temperatures is highlighted in bold.



Table 5. Performance of the best TVC PLS-R models developed using VIS-SWNIR (445 - 970 nm) and NIR (957 - 1664 nm) HSI data from beef *LD* samples stored at (i) 4 °C, (ii) 10 °C and (iii) either 4 °C or 10 °C using the optimum band selection method and LL, ML and HL data fusion

Regression model			Pre-treatment		#	#	Calibration			Cross validation			Prediction		
			1 <sup>st</sup>	2 <sup>nd</sup>	Bands	LV	RMSEc	RPDc	R <sup>2</sup> c	RMSEcv	RPDcv	R <sup>2</sup> cv	RMSEp	RPDp	R <sup>2</sup> p
<b>4 °C</b>															
VIS-SWNIR	EMCVS	log(1/R)	SNV	SD	6	5	0.88	2.83	0.88	1.03	2.42	0.83	1.17	1.88	0.83
NIR	EMCVS	log(1/R)	SD	AsLs	17	7	0.50	4.71	0.95	0.70	3.37	0.91	0.81	3.09	0.95
LL	VIP	R	SD	LD	22	8	0.76	3.28	0.91	1.09	2.30	0.81	1.16	1.90	0.88
ML						11	0.50	4.66	0.95	0.92	2.55	0.85	0.80	2.41	0.93
HL							<b>0.83</b>	<b>2.82</b>	<b>0.87</b>	<b>0.91</b>	<b>2.58</b>	<b>0.85</b>	<b>0.58</b>	<b>4.13</b>	<b>0.96</b>
<b>10 °C</b>															
VIS-SWNIR	EMCVS	R	SD	MS	46	6	<b>0.47</b>	<b>4.60</b>	<b>0.95</b>	<b>0.69</b>	<b>3.16</b>	<b>0.90</b>	<b>0.96</b>	<b>3.32</b>	<b>0.94</b>
NIR	EMCVS	log(1/R)	SNV		19	9	0.44	4.93	0.96	0.67	3.22	0.90	1.15	2.23	0.87
LL	EMCVS	log(1/R)	None		11	6	0.46	4.66	0.95	0.58	3.71	0.93	0.94	3.03	0.94
ML						6	0.47	4.64	0.95	0.75	2.89	0.88	1.17	2.17	0.84
HL							<b>0.36</b>	<b>6.07</b>	<b>0.97</b>	<b>0.51</b>	<b>4.30</b>	<b>0.95</b>	<b>0.97</b>	<b>3.28</b>	<b>0.94</b>
<b>4 °C and 10 °C</b>															
VIS-SWNIR	EMCVS	R	SNV	SD	8	4	0.94	2.48	0.84	1.05	2.22	0.80	0.95	2.10	0.85
NIR	EMCVS	R	SNV	SD	96	6	1.04	2.24	0.80	1.18	1.98	0.74	1.15	1.75	0.77
LL	EMCVS	R	SD	LD	35	4	<b>0.96</b>	<b>2.45</b>	<b>0.83</b>	<b>1.12</b>	<b>2.08</b>	<b>0.77</b>	<b>0.87</b>	<b>2.27</b>	<b>0.88</b>
ML						5	0.79	2.96	0.89	0.93	2.51	0.84	1.32	1.49	0.74
HL							<b>0.84</b>	<b>2.79</b>	<b>0.88</b>	<b>0.94</b>	<b>2.47</b>	<b>0.84</b>	<b>0.89</b>	<b>2.27</b>	<b>0.86</b>

EMCVS, ensemble Monte Carlo variable selection; VIP, variable importance projection ; SD, second derivative; SNV, standard normal variate; AsLs, asymmetric least squares; LD, linear detrend; MS, medium scaled: #Bands, wavelengths used for model development; #LVs, latent variables. The best model for each storage temperature is highlighted in bold.

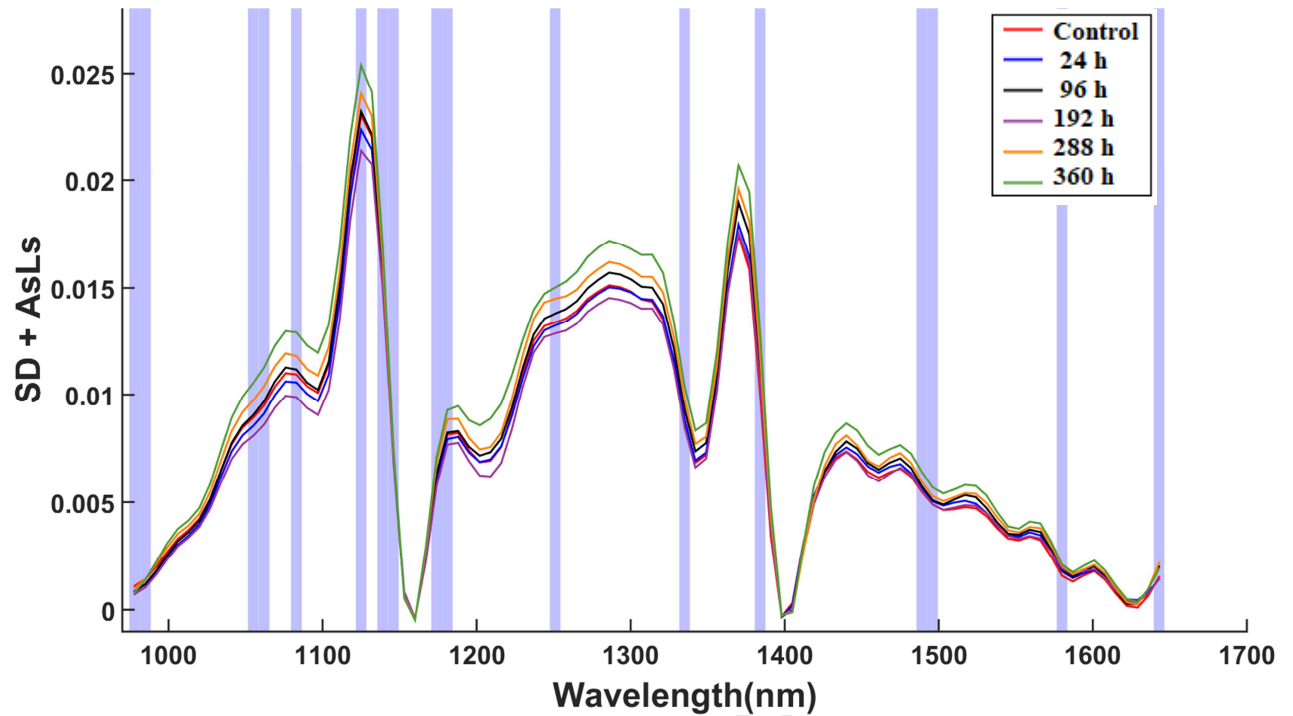


Fig. 1. Log (1/R) NIR pre-treated (SD+AsLs) spectra of beef *LD* samples stored at 4 °C for selected storage times. Bands selected by the EMCVS method to predict TVC of samples are highlighted in blue.

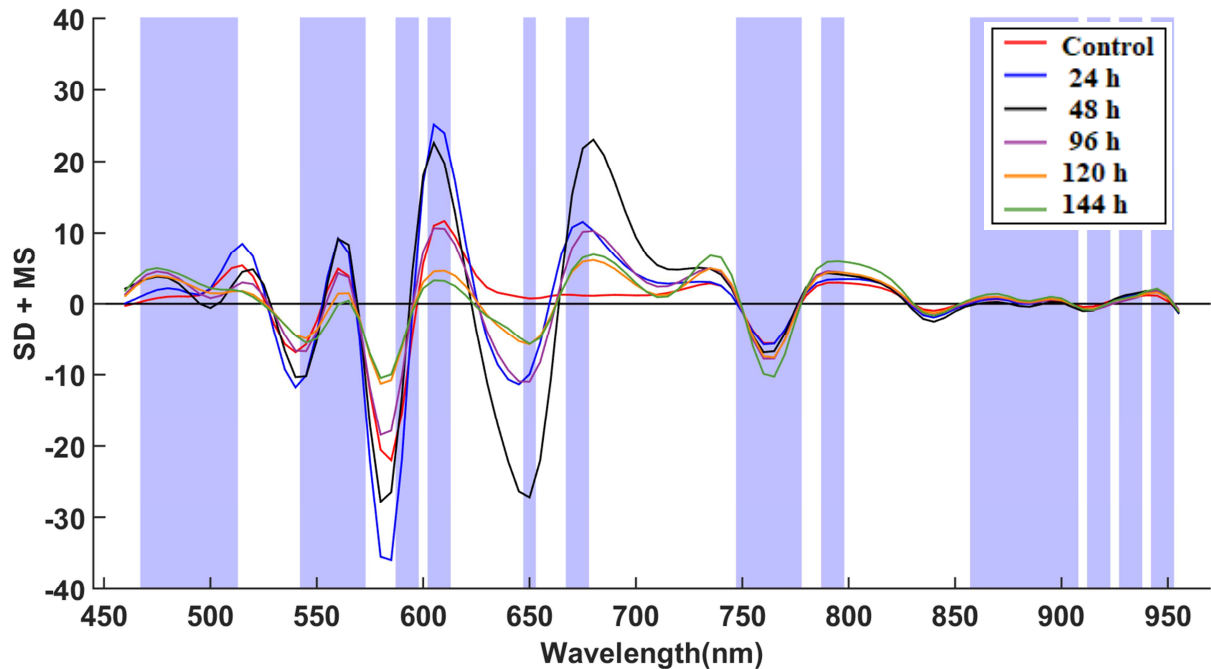


Fig. 2. Reflectance VIS-SWNIR pre-treated (SD+MS) spectra of beef *LD* samples stored at 10 °C for selected storage times. Bands selected by the EMCVS method to predict TVC of samples are highlighted in blue.

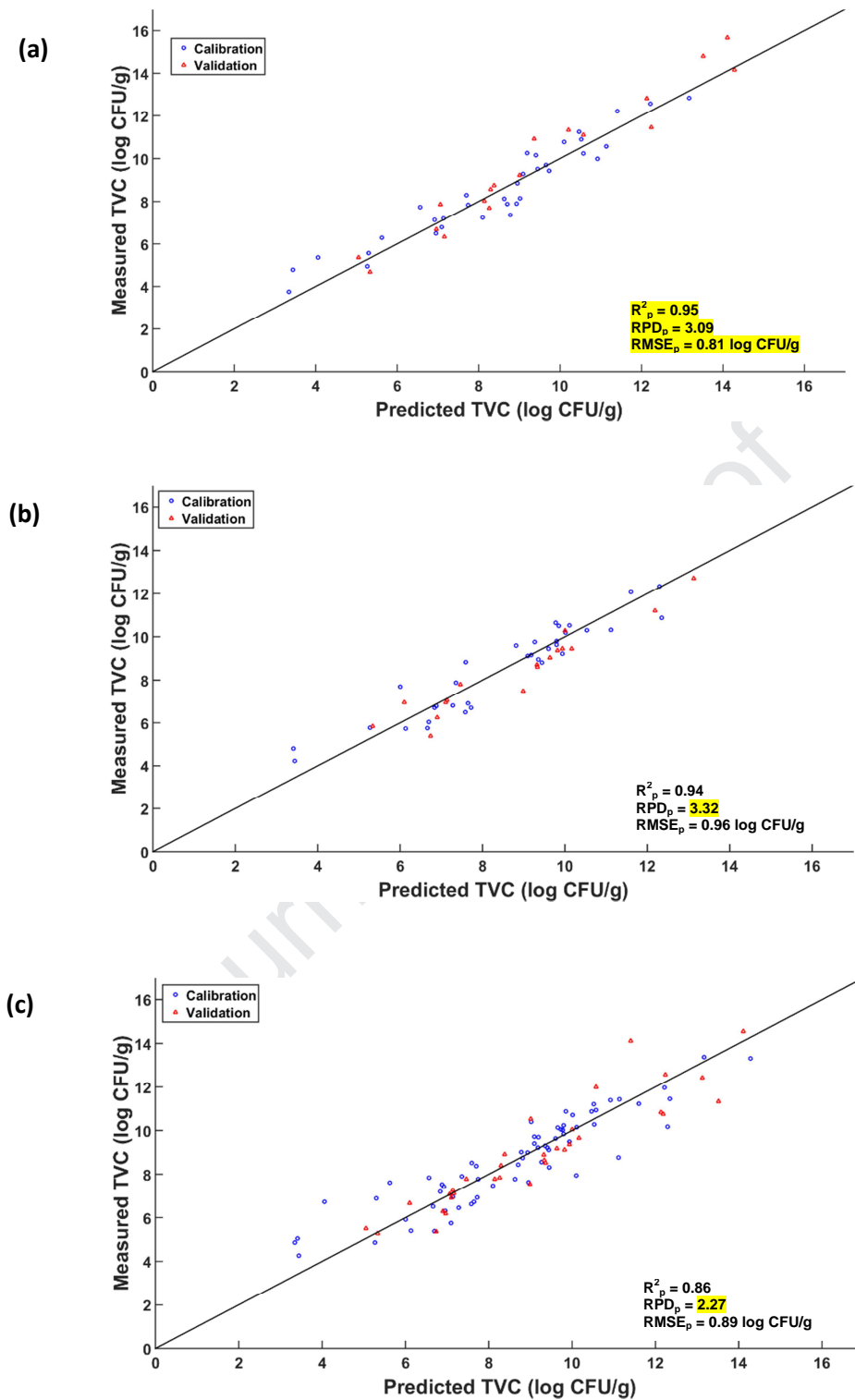


Fig. 3. Measured vs predicted TVC for the best performing PLS-R models developed for beef LD samples stored at (a) 4 °C, (b) 10 °C and (c) either 4 °C or 10 °C.

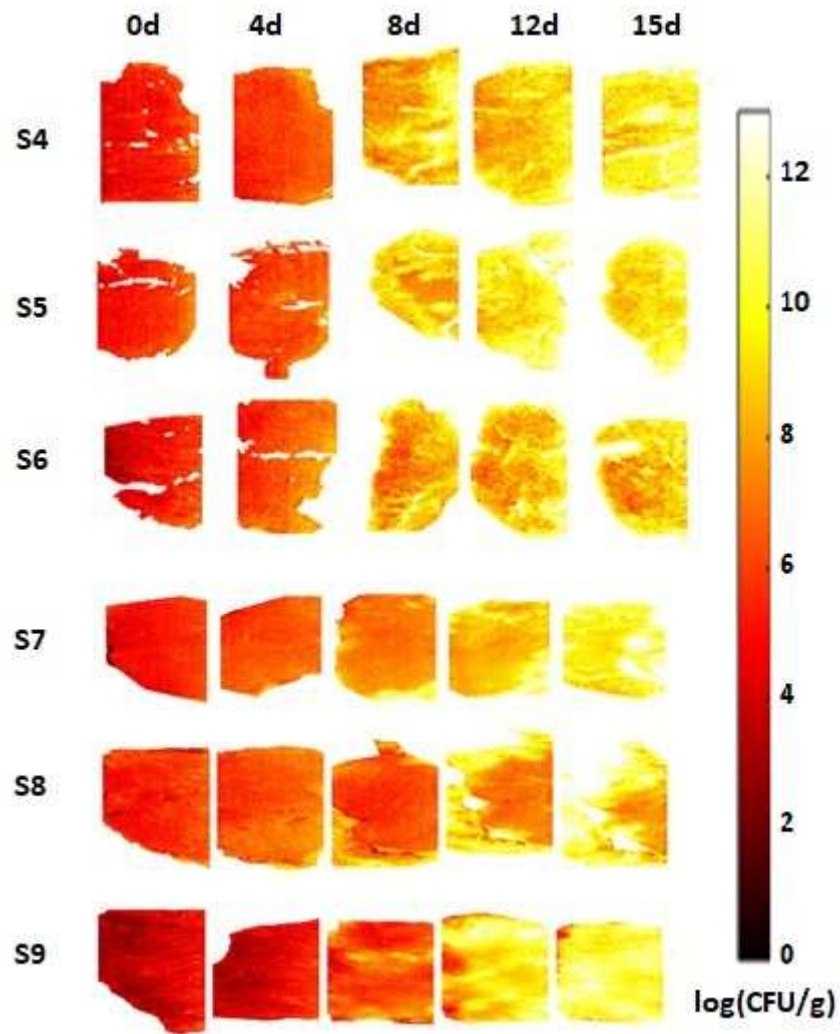


Fig. 4. Prediction maps for TVC of beef *LD* samples (log CFU/g) stored at 10 °C for selected times using the reflectance VIS-SWNIR pre-treated (SD+MS) spectra which selected 46 bands).

- Microbial quality of beef stored under normal or abuse conditions can be predicted
- Spectral pre-treatments, band selection and data fusion methods are key for robust model development
- Hyperspectral imaging and chemometrics have potential for real-time monitoring of microbial quality

## Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- ✓ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
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